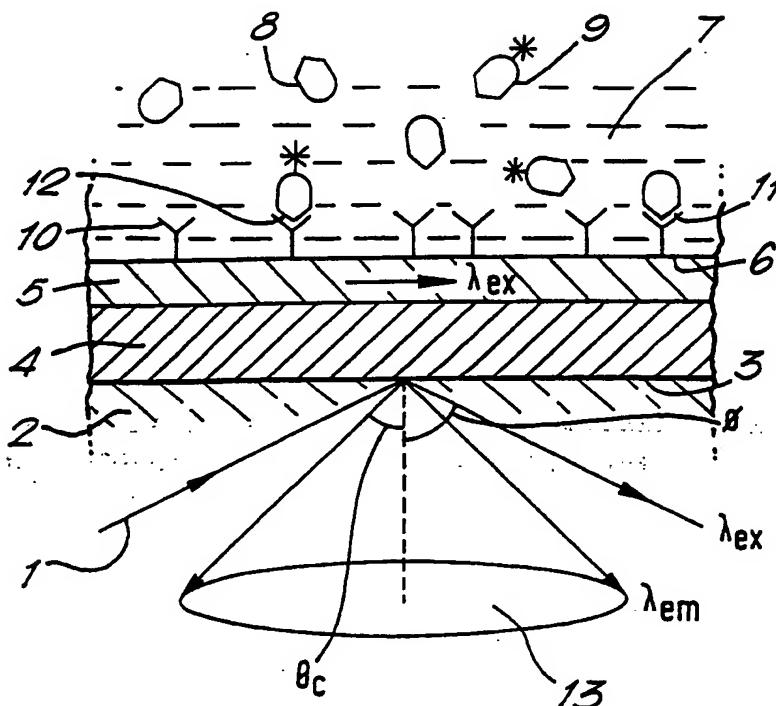




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁵ : G01N 21/77, 21/64, 33/53</p>	<p>A2</p>	<p>(11) International Publication Number: WO 90/06503 (43) International Publication Date: 14 June 1990 (14.06.90)</p>
<p>(21) International Application Number: PCT/GB89/01420 (22) International Filing Date: 28 November 1989 (28.11.89) (30) Priority data: 8827853.6 29 November 1988 (29.11.88) GB (71) Applicant (for all designated States except US): ARES-SER- ONO RESEARCH & DEVELOPMENT LIMITED PARTNERSHIP [GB/GB]; Exchange Place, 37th Floor, Boston, MA 02109 (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): ATTRIDGE, John, Wor- thington [GB/GB]; 135 Howbury Street, Bedford MK40 3QT (GB). (74) Agent: FRANK B. DEHN & CO.; Imperial House, 15-19 Kingsway, London WC2B 6UZ (GB).</p>		<p>(81) Designated States: AT (European patent), AU, BE (Euro- pean patent), CH (European patent), DE (European pa- tent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (Euro- pean patent), NL (European patent), NO, SE (European patent), US. Published <i>Without international search report and to be republished upon receipt of that report.</i></p>

(54) Title: SENSOR FOR OPTICAL ASSAY



(57) Abstract

The invention relates to a device for use in optical methods of assay. The device comprises an optical structure comprising a dielectric substrate (2) and a thin film waveguide (5), between which is interposed a buffer layer (4). The waveguide carries a layer (10) of reagent suitable for a desired assay. The invention also relates to methods of using the device in an assay.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MR	Mauritania
BE	Belgium	GA	Gabon	MW	Malawi
BF	Burkina Faso	GB	United Kingdom	NL	Netherlands
BG	Bulgaria	HU	Hungary	NO	Norway
BJ	Benin	IT	Italy	RO	Romania
BR	Brazil	JP	Japan	SD	Sudan
CA	Canada	KP	Democratic People's Republic of Korea	SE	Sweden
CF	Central African Republic	KR	Republic of Korea	SN	Senegal
CG	Congo	LI	Licchtenstein	SU	Soviet Union
CH	Switzerland	LK	Sri Lanka	TD	Chad
CM	Cameroon	LU	Luxembourg	TG	Togo
DE	Germany, Federal Republic of	MC	Monaco	US	United States of America
DK	Denmark				

Sensor for Optical Assay

This invention relates to a device for the enhancement of fluorescence and in particular to a device for use in fluorometric systems and methods for the assay of chemical or biochemical entities.

5 Methods of assay involving the use of fluorescence biosensors and employing evanescent wave coupling techniques are known, see for example EP-A-170376. The present invention provides means for carrying out assays employing evanescent wave coupling techniques whereby an
10 improved signal-to-noise ratio and enhanced sensitivity may be obtained.

US-4649280 describes a device for enhancing fluorescence comprising a waveguide defined by a dielectric layer of predetermined thickness having
15 layers of fluorescent and of conductive material on opposite surfaces of the said layer. The present invention avoids the need for a conductive layer, thereby reducing the cost of materials and increasing the ease of fabrication of the device. The invention
20 also allows greater design flexibility in producing sensors and allows a greater range of wavelengths of light to be used. Furthermore, methods according to the present invention are not polarisation dependant.

According to the present invention there is
25 provided a sensor device for use in assaying chemical or biochemical substances by optical methods which sensor comprises an optical structure comprising:

- (a) a dielectric substrate transparent at least at the wavelengths of radiation involved in the assay;
- 30 (b) a thin film waveguide of dielectric material having a refractive index higher than that of the substrate, the waveguide carrying a layer (continuous or discontinuous) of a reagent appropriate to the assay to

be carried out, the said reagent being immobilized directly or indirectly on the surface of the waveguide remote from the substrate; and

(c), interposed between (a) and (b), a buffer layer
5 of dielectric material of refractive index lower than that of the substrate; the thicknesses of the buffer layer and the waveguide being such that, in use, one or more guided modes may be propagated within the waveguide.

10 In a preferred embodiment, the thicknesses of the buffer layer and the waveguide are such that a single transverse magnetic (TM) and/or single transverse electric (TE) guided mode may be propagated within the waveguide.

15 A practical advantage accruing from use of an optical structure containing no metal layers is that fabrication of such devices is possible without the need for vacuum evaporation techniques; for example, spun-coated phosphate glasses may be used.

20 Furthermore, using a given combination of refractive indices of substrate, buffer layer, waveguide and solution it is possible in principle to design a device wherein the evanescent field is more highly confined than in previously known devices. When the
25 device is used in fluorometric methods of assay this therefore leads to better discrimination between fluorophores bound to the surface of the device and fluorophores in solution. Additionally, the intensity of the evanescent field is enhanced resulting in greater
30 excitation of the fluorophores within the evanescent field thereby improving the intensity of the emitted light compared to previously known devices.

Suitable materials for the substrate include glass, acrylic and polystyrene plastics, silica and quartz;
35 suitable materials for the buffer layer include any material of suitably low refractive index which can be formed as an optically transparent thin film, such as

magnesium fluoride, lithium fluoride, silicon dioxide and phosphate glasses; suitable materials for the waveguide layer include any material of suitably high refractive index which can be formed as an optically transparent thin film, such as zinc sulphide, zinc selenide, phosphate glasses lithium niobate, tin oxide and suitable polymers, e.g. polystyrene.

Preferably the buffer layer is 0.1 to 2 microns thick (for example, a buffer layer of SiO_2 may be 0.6 microns thick and a buffer layer of MgF_2 may be 0.7 microns thick) and the waveguide layer is 0.16 to 1.0 microns thick (for example, a waveguide layer of tin oxide may be 0.32 microns thick and a waveguide layer of ZnS may be 0.2 microns thick). The thickness of the substrate layer is not critical to the working of the sensor.

For maximum efficacy, the interfaces between the various layers of the sensor device should be smooth and parallel.

The sensors of the present invention are particularly suitable for use in methods of assay which are based on the affinity between the species which is to be assayed (hereinafter called "ligand") and a specific binding material for the ligand (hereinafter called "specific binding partner"). More particularly, the sensors of the invention can be used in assays involving measurement of fluorescence emitted by a fluorophore conjugated with an appropriate reagent.

The reagent appropriate to the assay to be carried out may be a specific binding partner for the ligand under assay or may be a ligand analogue. Examples of such entities are given later.

The sensors of the invention are also of use in assays involving measurement of absorption by a chromophore conjugated with an appropriate reagent.

According to a further feature of the present invention there is provided a method of using a sensor

as hereinbefore defined to assay for a ligand in a sample which method includes the steps of:

(a) incubating, simultaneously or in any desired sequence,

- 5 (i) the sample
- (ii) a specific binding partner to the ligand and
- (iii) if desired, at least one further reagent
 selected from specific binding partners and
 ligand analogues

10 one of the above components (other than the sample)
being the reagent immobilized directly or indirectly on
the waveguide surface of the said sensor; and at least
one of the above components (other than the sample)
being labelled with a fluorophore or a chromophore;

15 (b) irradiating a surface of the substrate of the said
sensor with light of an appropriate wavelength at a
suitable angle to the normal such that total internal
reflection occurs at the interface between the substrate
and the buffer layer of said sensor and whereby the
20 evanescent field associated therewith gives rise to
propagation of one or more guided modes in the waveguide
layer of said sensor; and

 (c) determining whether and, if desired, the extent to
which and/or rate at which an appropriate optical
25 property of radiation emerging from said sensor is
altered by complex formation. The optical property of
the light measured at a given angle to the plane of said
sensor may be, for example, wavelength, intensity or
polarization.

30 The alteration in the appropriate optical property
can, for example, be determined in accordance with step
(c) with reference to a separate calibration step, or by
incubating different zones of the device with sample and
reference solution(s) respectively.

35 The term "ligand analogue" as used herein means a
species which will bind specifically to the same binding
site of the same specific binding partner as will the

ligand and includes within its scope ligand molecules distinguishable from the ligand itself.

When the component (ii) (or one of components (ii) and (iii)) is labelled with a fluorophore, the radiation
5 of step (c) will be fluorescence radiation.

In a method of assay according to the invention involving measurement of absorption by a chromophore conjugated with an appropriate reagent, the assay and reagents used will be similar to the fluorometric assays
10 described above except that component (ii) (or one of components (ii) and (iii)) will be labelled with the chromophore instead of with a fluorophore and it will be an appropriate optical property of the reflected beam of radiation of the excitation wavelength which is
15 monitored during the assay. The invention will hereinafter be described with reference to fluorometric methods of assay.

Where component (iii) is absent, then the reagent immobilized directly or indirectly on the waveguide
20 surface of the sensor will be a specific binding partner labelled with a fluorophore. The reagent and fluorophore will be chosen such that on complex formation with any ligand present in the sample, quenching of fluorescence occurs such that there is a
25 decrease in intensity of fluorescence radiation.

Where component (iii) is present, then one of components (ii) and (iii) will be the reagent immobilized directly or indirectly on the waveguide surface of the sensor, denoted herein as reagent Y for
30 convenience, the other of components (ii) and (iii) being denoted herein for convenience as reagent X. Where one of the reagents X and Y comprises a ligand analogue and the other of reagents X and Y comprises a specific binding partner to the ligand, complex
35 formation between reagents X and Y may occur directly. Alternatively, where both reagents X and Y comprise specific binding partners to the ligand, complex

formation between reagents X and Y indirectly via the ligand, if present in the sample, may occur. In general, it will be reagent X which is labelled with the fluorophore and, as a result of complex formation, the fluorophore present as a label on reagent X becomes indirectly bound to the waveguide surface. However, if it is reagent Y which is labelled with fluorophore then with an appropriate choice of reagents and fluorophore, quenching of fluorescence can occur on complex formation.

Although the order of incubation is not critical when component (iii) is present, it is preferred that the complex is formed after introduction of the final component but not prior thereto. It is, however, alternatively possible for there to be complex present before the final component is added, in which case the final component will become complexed by displacing one component of the pre-existing complex. Where the final component is the sample and the component labelled with fluorophore is a ligand analogue, a displacement of ligand analogue by any ligand present in the sample will then result in a decrease in intensity of fluorescence radiation.

As mentioned above, reagent Y may be either directly or indirectly immobilized on the surface of the waveguide layer. For example, when reagent Y is an antibody, indirect immobilization may be effected by means of an anti-species antibody to reagent Y which is itself bound to the surface of said waveguide layer; alternatively indirect immobilization may be effected by means of avidin/biotin or hapten/anti-hapten systems.

The technique of the present invention makes it possible to maximise the response to complex formation at the surface of the sensor and when employed in fluorometric methods of assay significantly reduces the background signals arising from unbound fluorophore and from solution fluorescence.

In the sensors according to the present invention, the buffer layer and the waveguide layer will in general both have a thickness of the order of the wavelength of the incident radiation used. In particular, as

5 mentioned previously, the waveguide should be of a suitable thickness to support one or more guided modes but it is particularly preferred to employ a thickness of waveguide material which will support only a single guided TE or TM mode. For example, for a substrate of

10 refractive index 1.52 (e.g. crown glass) with a buffer layer of SiO_2 of thickness 0.6 micron and refractive index 1.46, and an incident radiation of wavelength 580 nm, the thickness of a waveguide layer of SnO_2 suitable for propagation of guided TE or TM modes

15 therein is approximately from 0.16 microns to 0.3 microns. Alternatively, for a substrate of crown glass (refractive index 1.52) with a buffer layer of MgF_2 of thickness 1 micron and refractive index 1.38 and an

20 incident radiation of wavelength 580 nm, the thickness of a waveguide layer of ZnS suitable for propagation of guided TE or TM modes therein is approximately 0.16 microns to 0.33 microns. It is important that the waveguide material has a high refractive index relative to that of the sample (e.g. 2.0 for SnO_2 ; 2.36 for ZnS)

25 in order that guiding can occur in a thin waveguide to maximise the excitation energy within the evanescent field.

The optics of the method of the present invention will now be discussed with reference to Fig. 1 which is

30 a schematic illustration of one possible sensor of the present invention in use. For simplicity, the lower boundary of the substrate layer (and any refraction of light which would occur thereat) is not shown.

Radiation (1) of a suitable wavelength λ_{ex} is applied to

35 the sensor through the substrate (2) such that it impinges on the interface (3) between the substrate (2) and the buffer layer (4) at an angle ϕ greater than the

critical angle θ_c between these layers, i.e. such that the exciting radiation is totally internally reflected and the evanescent field penetrates the buffer layer (4). As a consequence of evanescent coupling, radiation of the excitation wavelength λ_{ex} is propagated within the high index waveguide (5). With an appropriate choice of refractive indices and thicknesses for the substrate, buffer layer and waveguide layer, a significant enhancement of the evanescent field intensity is possible at the interface (6) of the waveguide and the sample solution (7). During the course of the particular type of assay illustrated by Fig. 1, free ligand molecules (8) and fluorescent ligand analogue molecules (9) in the solution compete for a particular binding site of a mutual specific binding partner (10) bound to the waveguide surface. As a result, complexes (11 and 12) are formed at the waveguide surface, some of which will contain fluorophores. The evanescent field decays exponentially away from the surface of the waveguide layer. Consequently, only fluorescently labelled molecules within the evanescent field at the waveguide surface become excited and fluoresce, thus achieving a discrimination between fluorophore bound to the surface as a result of the assay and the unbound fluorophore in solution. The fluorescence emission is markedly more intense than can be achieved by direct illumination using the same light source. A proportion of the fluorescence emission will couple back through the system in a known manner, to give a narrow angle cone (13) of emission of wavelength λ_{em} in the substrate, the angular distribution of which will depend on the waveguide dispersion and the fluorescence emission spectrum. The resulting enhanced narrow angle fluorescence emission can be measured in a known manner. The cone of emission shown in Fig. 1 is entirely schematic.

Enhanced fluorescence radiation is also emitted into the sample solution; the optical properties of this may also be measured. Alternatively, by a suitable arrangement of apparatus, detection of light emerging from an edge of the substrate or waveguide layer of said sensor may also be employed.

The present invention further provides apparatus suitable for use in the method of assay hereinbefore described which comprises a collimated source of radiation arranged such that, in use, light enters a sensor as described herein at an angle suitable to produce total internal reflection within the optical structure and means to detect the emergent fluorescence radiation. The exciting radiation may be collimated, for example, to within one or two degrees and may, in use, be introduced into a sensor within said apparatus, for example, through an edge of the substrate of the sensor or via a prism or a grating coupler. Ideally, the incident radiation is polarized, but unpolarized incident radiation may also be used.

According to a still further feature of the present invention there is provided a kit for use in an assay as described above, the said kit comprising a sensor according to the invention as hereinbefore defined together with one or more reagents appropriate to the assay to be carried out. The reagents may be selected from specific binding partners and ligand analogues.

It is preferred to apply the method of the invention to an immunoassay and in particular to use a specifically-reactive sample collecting and testing device similar to that described in EP-0171148. Thus, the present invention provides a device having a cavity or cavities each having a dimension small enough to enable sample liquid to be drawn into the cavity by capillary action, and wherein at least one part of a wall of said cavity comprises a sensor for detecting a ligand in said sample as defined hereinbefore. In this

particular embodiment of the invention the substrate itself comprises a planar waveguide.

The method of the invention is particularly applicable to assays of antigens or antibodies, i.e. to immunoassays and in a preferred embodiment of the invention the ligand is an antigen and the specific binding partner comprises an antibody to the said antigen. However, the invention is not to be taken as limited to assays of antibodies or antigens. As mentioned above, a reagent immobilized on the waveguide surface of a sensor according to the present invention will be either a specific binding partner to the ligand under assay or a ligand analogue. Examples of ligands which may be assayed by the method of the invention are given in Table 1 below, together with an indication of a suitable specific binding partner in each instance.

Table 1

5	Ligand	Specific Binding Partner
	antigen	specific antibody
	antibody	antigen
	hormone	hormone receptor
10	hormone receptor	hormone
	polynucleotide strand	complementary polynucleotide strand
	avidin	biotin
	biotin	avidin
15	protein A	immunoglobulin
	immunoglobulin	protein A
	enzyme	enzyme cofactor (substrate) or inhibitor
20	enzyme cofactor (substrate) or inhibitor	enzyme
	lectins	specific carbohydrate
	specific carbohydrate of lectins	lectins

25

The method of the invention has very broad applicability but in particular may be used to assay: hormones, including peptide hormones (e.g. thyroid stimulating hormone (TSH), luteinizing hormone (LH), human chorionic gonadotrophin (hCG), follicle stimulating hormone (FSH), insulin and prolactin) or non-peptide hormones (e.g. steroid hormones such as cortisol, estradiol, progesterone and testosterone, or thyroid hormones such as thyroxine (T4) and triiodothyronine), proteins (e.g. carcinoembryonic antigen (CEA) and alphafetoprotein (AFP)), drugs (e.g. digoxin or drugs of abuse), sugars, toxins, vitamins, proteins, viruses such as influenza, para-influenza,

30

35

adeno-, hepatitis, respiratory and AIDS viruses, or microorganisms.

It will be understood that the term "antibody" used herein includes within its scope:

- 5 (a) any of the various classes or sub-classes of immunoglobulin, e.g. IgG, IgA, IgM, or IgE derived from any of the animals conventionally used, e.g. sheep, rabbits, goats or mice,
- (b) monoclonal antibodies,
- 10 (c) intact molecules or "fragments" of antibodies, monoclonal or polyclonal, the fragments being those which contain the binding region of the antibody, i.e. fragments devoid of the Fc portion (e.g. Fab, Fab', F(ab')₂) or the so-called "half-molecule"
- 15 fragments obtained by reductive cleavage of the disulphide bonds connecting the heavy chain components in the intact antibody.

The method of preparation of fragments of antibodies is well known in the art and will not be
20 described herein.

The term "antigen" as used herein will be understood to include both permanently antigenic species (for example, proteins, bacteria, bacterial fragments, cells, cell fragments and viruses) and haptens which may be
25 rendered antigenic under suitable conditions.

Examples of fluorescent molecules which may be used to label the ligand conjugate are fluorescein isothiocyanate (FITC), rhodamine isothiocyanate, 2,4-dinitrofluorobenzene, phenylisothiocyanate and
30 dansyl chloride, XRITC, TRITC, tetramethylrhodamine codaverine (TRAP) and phycobiliproteins (e.g. allophycocyanin and phycoerythrin).

The following non-limiting Examples illustrate the construction of sensors in accordance with the invention
35 and assays using such sensors.

Example 1Construction of Sensors

A suitable substrate material (e.g. crown glass, refractive index 1.52) is cleaned and a buffer layer of silicon dioxide (refractive index 1.46), 0.6 microns thick, is deposited onto the glass substrate by vacuum evaporation techniques known in the art. A layer of tin (IV) oxide (refractive index 2.0), 0.3 microns thick, is then deposited onto the magnesium fluoride, also by vacuum evaporation. The layer of tin (IV) oxide is to act as the waveguide part of the sensor.

Antibody specific for the antigen of interest is immobilized on the surface of the waveguide, either by adsorption or by covalent coupling using techniques known in the art.

An alternative structure would comprise a magnesium fluoride buffer layer (refractive index 1.38), 0.7 microns thick, and a tin oxide waveguide, 0.25 microns thick.

Alternative materials for the waveguide are zinc sulphide (refractive index 2.36) as a layer 0.2 microns thick, which can be deposited onto the magnesium fluoride layer by vacuum evaporation; and an indium phosphate glass (refractive index 1.61) as a layer 0.5 microns thick, which can be deposited onto the magnesium fluoride layer by spin coating. The refractive indices of indium phosphate glasses can be precisely controlled (see Sloper and Flanagan, Electronics Letters 24 (1988)).

The embodiments described in this Example are each capable of enhancing the electric field intensity at the surface of the waveguide by up to 260 times, when used in a method according to the invention, compared with a method employing direct illumination of the surface.

Example 2Use of a sensor of Example 1 in an assay for an antigen

The appropriate surface of a sensor as described in
5 Example 1 is incubated with sample liquid and
(simultaneously or sequentially) with fluorescently
labelled antigen or a fluorescently labelled second
antibody specific for the antigen of interest, depending
upon the chosen assay methodology. As a result, if the
10 antigen under assay is present in the sample, immuno-
complexes are formed on the waveguide surface of the
sensor, whereby fluorophores become bound to the
waveguide surface.

A surface of the substrate is irradiated at a
15 suitable wavelength and angle to excite a guided mode
within the waveguide. Any fluorophores within the
evanescent field at the waveguide surface are excited by
the enhanced evanescent wave associated with this guided
mode. Preferred sources of radiation are of
20 monochromatic and/or polarised light (e.g. from a
laser).

Fluorescence radiation which is emitted is
monitored. Light emerging from the sensor is collected
by the detection optics and any light of the excitation
25 wavelength is preferably filtered out before the
intensity measurement is made. The fluorescent light
emerging from the substrate which originates from any
fluorophores bound to the waveguide surface has a
distinct angular distribution (see Fig. 1). The
30 operating signal-to-noise ratio of the sensor is thus
very low, particularly when the radiation source is
polarized.

The detection optics will consist of a
photodetector (either a photomultiplier tube or a solid
35 state detector) with, optionally, some filtration to
remove light of excitation wavelength; examination of
the polarisation of the light may be beneficial and may

therefore optionally be performed. Use of an aperture also facilitates ease of detection.

In an alternative method of assay according to the invention, the fluorophores may be excited by direct
5 illumination, either through the substrate or through the sample, such that the emitted light is collected via the guided modes of the device. In this embodiment, which is analogous to the method described in EP-170376, the enhanced intensity of the evanescent field is lost
10 but the signal to noise ratio of the device is improved via the reduced angular distribution of the emitted light which allows for increased efficiency of collection and measurement. According to a further feature of the present invention there is thus provided
15 a kit for use in a method of assay as described above comprising a sensor as described above together with one or more reagents appropriate to the said method.

Claims:

1. A sensor for use in optical methods of assay which comprises an optical structure comprising:
 - 5 (a) a dielectric substrate transparent at least at the wavelengths of radiation involved in the assay;
 - (b) a thin-film waveguide of dielectric material having a refractive index higher than that of the substrate, the waveguide carrying a layer
10 (continuous or discontinuous) of a reagent appropriate to the assay to be carried out, the said reagent being immobilised directly or indirectly on the surface of the waveguide remote from the substrate; and
 - 15 (c) interposed between (a) and (b), a buffer layer of dielectric material of refractive index lower than that of the substrate;the thicknesses of the buffer layer and the waveguide being such that, in use, one or more guided modes may be
20 propagated within the waveguide.
2. A sensor as claimed in claim 1 wherein the thicknesses of the buffer layer and the waveguide are such that, in use, a single transverse magnetic (TM)
25 and/or transverse electric (TE) guided mode may be propagated within the waveguide.
3. A sensor as claimed in claim 1 or claim 2 wherein the buffer layer has a thickness of between 0.1 and 2.0
30 microns and the waveguide has a thickness of between 0.16 and 1.0 microns.
4. A sensor as claimed in claim 3 wherein the buffer layer is of silicon dioxide and has a thickness of about
35 0.6 microns and the waveguide is of tin oxide and has a thickness of about 0.3 microns.

5. A sensor as claimed in claim 3 wherein the buffer layer is of magnesium fluoride and has a thickness of about 0.7 microns and the waveguide is of zinc sulphide and has a thickness of about 0.2 microns.

5

6. A method of using a sensor as claimed in any one of claims 1 to 5 in an assay for a ligand in a sample which method includes the steps of:

- 10 (a) incubating, simultaneously or in any desired sequence,
- (i) the sample
- (ii) a specific binding partner to the ligand and
- 15 (iii) if desired, at least one further reagent selected from specific binding partners and ligand analogues,
- one of the above components (other than the sample) being the reagent immobilized directly or
- 20 indirectly on the waveguide surface of the said sensor, and at least one of the above components (other than the sample) being labelled with a fluorophore or a chromophore;
- (b) irradiating a surface of the substrate of the said
- 25 sensor with light of an appropriate wavelength at a suitable angle to the normal such that total internal reflection occurs at the interface between the substrate and the buffer layer of said sensor and whereby the evanescent field associated
- 30 therewith gives rise to propagation of one or more guided modes in the waveguide of said sensor; and
- (c) determining whether and, if desired, the extent to which and/or rate at which an appropriate optical property of radiation emerging from said sensor is
- 35 altered by complex formation.

7. A method as claimed in claim 6 wherein, in step

(a), at least one of components (ii) and (iii) is labelled with a fluorophore and, in step (c), the said radiation is fluorescence radiation.

- 5 8. A method as claimed in claim 6 wherein, in step (a), at least one of components (ii) and (iii) is labelled with a chromophore.
9. A method as claimed in claim 6 wherein component
10 (iii) is present as a ligand analogue possessing a fluorophore as a label.
10. A method as claimed in any one of claims 6 to 9 wherein the ligand is an antigen and the specific
15 binding partner comprises an antibody to the said antigen.
11. A specifically reactive sample collecting and testing device possessing a cavity or cavities each
20 having a dimension small enough to enable sample liquid to be drawn into the cavity by capillary action, and wherein at least one part of a wall of said cavity comprises a sensor as claimed in any one of claims 1 to 5.
- 25 12. Apparatus for use in a method of assay as claimed in any one of claims 6 to 10 which comprises a sensor as claimed in any one of claims 1 to 5, a collimated source of radiation arranged such that, in use, it enters the
30 sensor at an angle suitable to produce total internal reflection within the optical structure and means to detect the emergent radiation.
13. A kit for use in a method of assay as claimed in
35 any one of claims 6 to 10 comprising a sensor as claimed in any one of claims 1 to 5 together with one or more reagents appropriate to the said method.

14. A method of using a sensor as claimed in any one of claims 1 to 3 in an assay for a ligand in a sample which method includes the steps of:

- 5 (a) incubating, simultaneously or in any desired sequence,
 (i) the sample
 (ii) a specific binding partner to the ligand and
 (iii) if desired, at least one further reagent
10 selected from specific binding partners and ligand analogues,
 one of the above components (other than the sample) being the reagent immobilized directly or indirectly on the waveguide surface of the said
15 sensor as claimed in any one of claims 1 to 3, and at least one of the above components (other than the sample) being labelled with a fluorophore;
 (b) irradiating the sample with light of an appropriate wavelength to excite the fluorophores whereby light
20 emitted from fluorophores located within the evanescent field couples into a guided mode within the waveguide; and
 (c) determining whether and, if desired, the extent to which and/or rate at which an appropriate optical
25 property of radiation emerging from said sensor is altered by complex formation.

1/1

FIG. 1.

